



## Western Blotting

Some protein detection kits  
make you wonder if your eyes  
are playing tricks on you.



SEE MORE with KPL!



# Western Blotting

**No matter which KPL Western Blotting system you use, you'll get a strong, clean signal every time.**

KPL's blotting substrates and Protein Detector™ Western Blot Kits provide consistent, high quality results when analyzing proteins immobilized on membrane. Choose from a line of substrates and kits for either chemiluminescent or colorimetric methods of detecting specific antigens in a complex protein mixture.

The cornerstone of KPL's Western blotting systems is our line of sensitive liquid substrates. You have a choice among multiple chemiluminescent and chromogenic substrates

for peroxidase and phosphatase that provide varied levels of sensitivity. The chemiluminescent substrates provide a reliable alternative to conventional colorimetric ELISA with the advantage of increased sensitivity.

Our chemiluminescent substrates for alkaline phosphatase detection, PhosphaGLO™ and PhosphaGLO Reserve™ AP Chemiluminescent Substrates, offer sensitivity and duration of signal in a one-component system.

KPL's substrates are offered as stand-alone reagents or combined with unique blocking and wash solutions and highly specific secondary antibodies to create comprehensive, fully optimized kits for Western blotting. Each of these kits is designed to deliver the maximum signal-to-noise ratio available. Choose the detection system that best meets your needs and achieve clean, clear results blot after blot.

## Substrates for Western Blotting

KPL's Western blotting substrates for use in peroxidase and phosphatase reporter systems offer high quality, reproducible chemiluminescent and colorimetric detection. It's your choice!

	<b>LumiGLO Reserve™ HRP Substrate</b>	<b>LumiGLO® HRP Substrate</b>	<b>PhosphaGLO Reserve™ AP Substrate</b>	<b>PhosphaGLO™ AP Substrate</b>	<b>TMB HRP Substrate</b>	<b>4 CN Substrate</b>	<b>BCIP/NBT AP Substrate</b>	<b>FirePhos™ Membrane AP Substrate</b>
Type	chemi.	chemi.	chemi.	chemi.	color.	color.	color.	color.
Format	2-comp	2-comp	1-comp	1-comp	1- or 3-comp	2-comp	1- or 3-comp	1-comp
Detection Method	film or image analysis	film or image analysis	film or image analysis	film or image analysis	visual-dark blue precipitate	visual - purple precipitate	visual - purple precipitate	visual - red
Detection Limit	Sub-picogram; femtogram	0.6 picogram	femtogram	picogram	50 picogram	500 picogram	low nanogram	low nanogram
Stability of Working Solution	8 hours	24 hours	2 years at 4° C	2 years at 4° C	1-comp-1 year at 4° C. 3-comp-24 hours	1 hour	1-comp-1 year at 4° C. 3-comp-1 hour	NA
Duration of Signal	4 - 8 hours	1 - 2 hours	5 days	5 days	NA	NA	NA	NA
Enzyme Catalyst	HRP	HRP	AP	AP	HRP	HRP	AP	AP
Kinetics	5 minutes	5 minutes	15 minutes	15 minutes	10 minutes	10 minutes	10 minutes	10 minutes
Recommended Membrane	nitrocellulose or PVDF	nitrocellulose or PVDF	nitrocellulose or PVDF	nitrocellulose or PVDF	nitrocellulose or PVDF	nitrocellulose or PVDF	nitrocellulose or PVDF	nitrocellulose or PVDF

comp = component • chemi. = chemiluminescent • color. = colorimetric • HRP = peroxidase • AP = phosphatase • NA = not applicable





With KPL's Protein Detector™  
Western Blotting Systems, the  
results jump right out at you.



## Chemiluminescent Substrates and Western Blot Kits

### Rapid, Sensitive Detection with Luminol-based Substrates

Chemiluminescent detection involves the creation of light through the catalysis of an enzyme substrate. Use of this method for protein detection allows an increase in sensitivity by orders of magnitude compared to traditional colorimetric Western or dot blotting. KPL offers two unique luminol-based chemiluminescent substrates —LumiGLO® and LumiGLO Reserve™, for the rapid and sensitive detection of horseradish peroxidase (HRP)-labeled conjugates.

### Maximum Sensitivity with LumiGLO Reserve™

LumiGLO Reserve HRP Chemiluminescent Substrate offers an option for those assays where enhanced sensitivity is critical to success. This proprietary two-component substrate formulation provides greater than 20 times the sensitivity of standard LumiGLO with detection levels as low as the femtogram range (Figure 1).

### Long Signal Duration

LumiGLO Reserve emits light over the course of 4-8 hours with the most intense emission occurring within the first two hours. Because of its extreme light intensity, most images may be captured well under 10 minutes; multiple exposures are easy to obtain. High signal intensity facilitates the detection of both high and low abundance proteins and makes it an ideal system for use with chemiluminescent imagers.

### Sample and Antibody Conservation

LumiGLO Reserve provides the added benefit of strong signal with the use of reduced amounts of precious target and antibodies. Therefore, material of limited supply or higher expense can be conserved while maintaining your current level of sensitivity.

### Superior Signal to Noise

LumiGLO Reserve delivers lower non-specific signal than competitor substrates in its class (Figures 2 and 3).

### Convenience

LumiGLO Reserve Chemiluminescent Substrate Kits are supplied in several kit formats to meet diverse user needs. This substrate can replace your current chemiluminescent substrate in existing assays where greater sensitivity is desired.

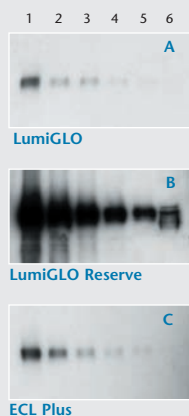
### LumiGLO Reserve Western Blot Kit

LumiGLO Reserve is also offered as part of a fully optimized kit, the Protein Detector LumiGLO Reserve Western Blot Kit, providing femtogram detection of proteins immobilized on membranes. For assurance against background interference, this kit contains our unique Detector™ Block for optimal signal-to-noise ratio; very low background can be achieved without compromise to signal intensity (Figures 2 and 3). Detector Block is also offered separately.

### Reliable and Economical LumiGLO®

KPL's original LumiGLO Chemiluminescent Substrate provides high quality results in a variety of immunoassays.

## LumiGLO Reserve Signal-to-Noise Comparison



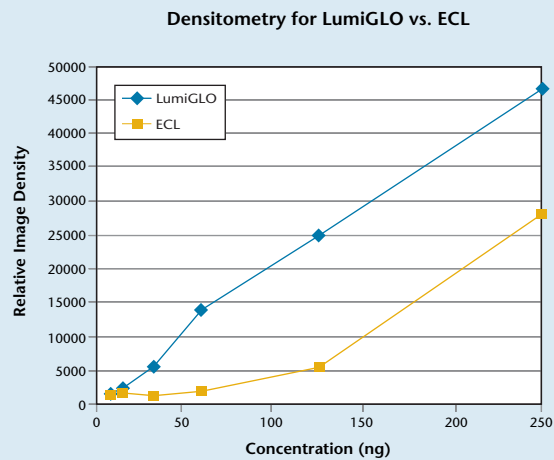
**Figure 1:** Relative expression of transcription factor, c-myc, using different chemiluminescent substrates. Five two-fold serial dilutions of purified c-myc (25 ng–1.56 ng, lanes 1–5) were compared to a 64 µg total protein HeLa nuclear lysate (lane 6). Following separation on a 4-20% PAGE gel and transfer to PVDF, protein was detected using a rabbit anti-c-myc antibody (1:200) and anti-rabbit HRP conjugate (1:10,000). Detection conditions were identical with the exception of substrate.

While the c-myc lysate sample was not detectable with A) LumiGLO or C) ECL Plus™ after 10 minutes, the sample was easily detected with B) LumiGLO Reserve after just a 2-minute film exposure.



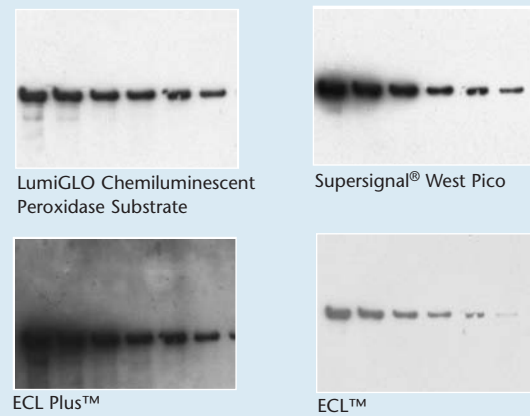
**Figure 2:** Comparison of low-end sensitivity using LumiGLO Reserve and ECL Detection Kits. Two-fold serial dilutions of Mouse IgG (1 ng – 31 µg) were separated by SDS-PAGE and transferred to PVDF. Under manufacturer's recommended conditions, protein was detected using HRP-labeled anti-mouse antibody (varied dilutions according to recommended optimization) and each respective substrate: A) LumiGLO Reserve, B) ECL Plus™, C) ECL Advance™. Film was exposed for 10 minutes and analyzed for sensitivity and signal to noise.

## LumiGLO vs. Leading Traditional Chemiluminescent Substrates



**Figure 4:** Signal response comparison of LumiGLO vs. ECL™ chemiluminescent substrates in Western blotting.  $\beta$ -galactosidase was electrophoresed, transferred to PVDF membrane and subsequently detected using rabbit anti- $\beta$ -gal followed by HRP conjugated goat anti-rabbit IgG (H+L). Each blot was treated with 5 mL of LumiGLO or ECL substrate and exposed to film for 10 minutes. The density of each band was analyzed on a Syngene GeneGenius™ image analyzer, using automatic background subtract.

### Superior Signal to Noise with Detector™ Block



**Figure 5:** Detection of  $\beta$ -galactosidase in Western blots using alternative chemiluminescent substrates. A two-fold dilution series of purified  $\beta$ -galactosidase (from 25  $\mu$ g) was electrophoresed on a polyacrylamide gel and transferred to KODAK BIOMAX Multi-Blot Kit for Proteins. Each blot was detected under the same conditions using Protein Detector LumiGLO Western Blotting Kit, substituting 5 mL LumiGLO on 3 of the 4 blots with 5 mL of leading competitive chemiluminescent substrates, respectively. Following a 10 minute film exposure, results were evaluated for sensitivity and signal:noise ratio.

Researchers continue to select LumiGLO for reliable performance in Western blot detection.

### Ideal for Routine Western Blotting

LumiGLO detects low picogram quantities of target protein on blots. After reaction with membrane-bound HRP conjugates, light emission begins immediately and continues for approximately 1–2 hours. Detection is possible within minutes of blot exposure.

### Greater Linearity

LumiGLO produces a quantitatively linear signal on film that ensures a broader dynamic range of detection. While the light output from other chemiluminescent substrates tends to reduce sharply as the concentration of protein is titrated, a proportional reduction in sensitivity is achieved with LumiGLO (Figure 4).

### Low Background

LumiGLO delivers superior signal-to-noise by design. When used with KPL's Detector™ Block, nonspecific binding is

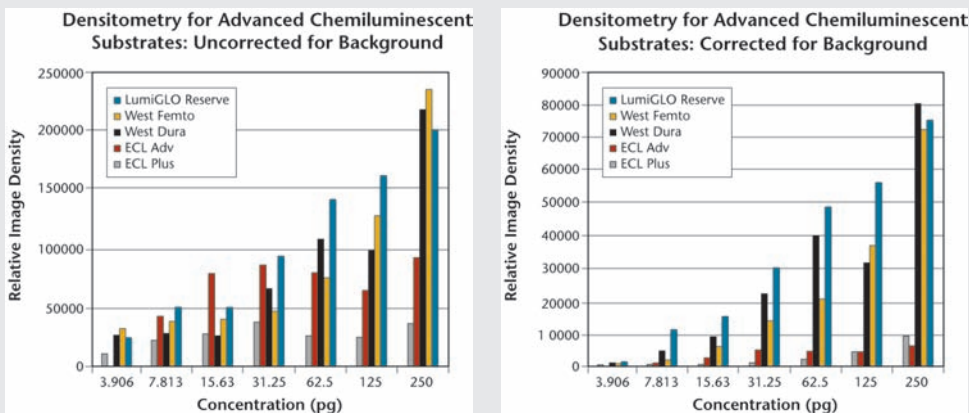
further reduced and ensures low background without loss of signal intensity. Figure 5 compares the superior signal-to-noise from blots blocked with Detector Block and subsequently detected with LumiGLO to leading competitor substrates.

### Cost Effective

LumiGLO is more economical blot for blot compared to competitive products. Enjoy the benefits of a sensitive, reliable substrate while staying within budget.

### LumiGLO Western Blot Kit

The LumiGLO Western Blot Kit is designed for low picogram detection of proteins immobilized on membranes. Researchers choose this kit for its reliable, consistent performance. The combination of a stable, liquid conjugate and a sensitive chemiluminescent substrate allows rapid and accurate identification of samples. For assurance against background interference, this kit contains our unique Detector Block for optimal signal-to-noise ratio (Figure 5).



**Figure 3:** Signal linearity and signal-to-noise ratio comparison of advanced chemiluminescent substrates. Mouse IgG (250 pg to 3.91 pg) was transferred to nitrocellulose. Blots were detected under identical conditions with a 1:10,000 dilution of HRP-Goat-anti-Mouse IgG (except ECL Advance, 1:100,000) and exposed to film for 10 minutes. Densitometry was then performed using the Syngene GeneGenius. LumiGLO Reserve demonstrates superior signal linearity over a larger dynamic range and the greatest comparative signal-to-noise.

# Western Blotting

## Sensitivity and Convenience with PhosphaGLO™ AP Substrates

KPL offers two chemiluminescent substrates for use with alkaline phosphatase, PhosphaGLO Reserve™ and PhosphaGLO™ AP Substrates. PhosphaGLO Reserve Substrate overcomes the limitations posed by conventional chemiluminescent substrates for AP. With sensitivity in the femtogram range, PhosphaGLO Reserve enables detection of target protein in low concentrations. PhosphaGLO AP Substrate is recommended for routine detection of proteins in the picogram range (Figure 6).

## Low Background without Special Blockers

PhosphaGLO Substrates produce superior signal and low background, providing a better signal to noise ratio and a cleaner image than other chemiluminescent substrates for AP (Figure 6). No special blockers are needed with either nitrocellulose or PVDF membrane.

## Ultimate Convenience

Both PhosphaGLO AP Substrates offer exceptional convenience as one-component solutions ready to use. They are stable for up to two years when stored at 4° C. Their long glow times of 5 days facilitate assay development, enabling repeat exposures.

## Colorimetric Kits

KPL also offers colorimetric Western blot systems when chemiluminescent detection is not preferred. Results can be interpreted by direct visualization of the blot. Everything you need to detect your antigen and primary antibody is supplied for convenience and optimal performance.

## Protein Detector™ TMB Western Blot Kit

The Protein Detector™ TMB Western Blot Kit utilizes TMB Peroxidase Membrane Substrate (3,3',5,5'-tetramethylbenzidine), the most sensitive chromogenic peroxidase substrate for Western and dot blotting applications. Detection limits are significantly increased as compared to other chromogenic membrane substrates. TMB produces a dark blue precipitate upon reaction with HRP.

## Protein Detector™ BCIP/NBT Western Blot Kit

For detection of phosphatase-labeled conjugates, the Protein Detector BCIP/NBT Western Blot Kit is ideal. When reacted with alkaline phosphatase, BCIP/NBT produces clean, intense bands of purple precipitate. It also provides stable, more permanent results than other chromogenic substrates.

## Western Blot Reagents

In addition to our line of substrates and kits, KPL offers a broad line of secondary antibodies, conjugates and support reagents in various packaging formats to suit your needs.

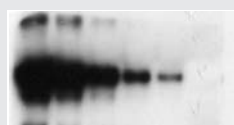
### Secondary Antibodies

- Highly purified and specific.
- Large offering of species-specific polyclonal antibodies
- Enzyme, biotin and fluorochrome-labeled antibodies

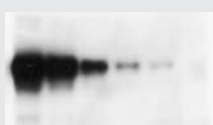
### Support Reagents

- One source for block solutions, wash buffers, diluents and stabilizers.
- Consistently produced for reliable results.

PhosphaGLO Reserve AP Substrate



PhosphaGLO AP Substrate



CDP Star® Chemiluminescent Substrate



Figure 6: Comparison of low-end sensitivity using PhosphaGLO Reserve AP Substrate, PhosphaGLO AP Substrate, and CDP-Star Chemiluminescent Substrate on PVDF membranes. Five-fold serial dilutions of mouse IgG (2 ng -3.2 pg) were separated by SDS-PAGE and transferred to membranes. Protein was detected using a 1:1000 dilution of biotin-labeled goat anti-mouse IgG, a 1:10,000 dilution of AP-labeled streptavidin and each respective substrate. Film was exposed for 10 minutes.

LumiGLO is a registered trademark and LumiGLO Reserve, Protein Detector, PhosphaGLO, PhosphaGLO Reserve, FirePhos and Detector are trademarks of KPL, Inc.

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SuperSignal West is a registered trademark of Pierce Biotechnology, Inc.

BIOMAX is a trademark of KODAK.

GeneGenius is a trademark of Syngene.

Biodyne is a registered trademark of Pall/Gelman Corporation.

CDP-Star is a registered trademark of Applied Biosystems.

Catalog #	Description	Size
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### Protein Detector™ Western Blotting Kits

Each kit includes anti-mouse and anti-rabbit conjugates, Detector Block, Wash Solution Concentrate and Substrate.

#### Phosphatase Chromogenic

55-11-50	BCIP/NBT Western Blot Kit	2500 cm <sup>2</sup>
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#### Peroxidase Chromogenic

54-11-50	TMB Western Blot Kit	2500 cm <sup>2</sup>
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#### Peroxidase Chemiluminescent

54-12-50	LumiGLO® Western Blot Kit	2500 cm <sup>2</sup>
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54-13-50	LumiGLO Reserve™ Western Blot Kit	2400 cm <sup>2</sup>
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### Related Reagents and Kits

#### Antibody Conjugates

All antibodies listed below are produced in goat. For a complete antibody listing, refer to KPL's Product Catalog.

#### Phosphatase-labeled

475-1006	Anti-Human IgG (H+L)	1.0 mL, liquid
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475-1806	Anti-Mouse IgG (H+L), HSA	1.0 mL, liquid
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475-1506	Anti-Rabbit IgG (H+L)	1.0 mL, liquid
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#### Peroxidase-labeled

474-1006	Anti-Human IgG (H+L)	1.0 mL, liquid
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474-1806	Anti-Mouse IgG (H+L) HSA	1.0 mL, liquid
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474-1506	Anti-Rabbit IgG (H+L)	1.0 mL, liquid
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#### Biotin-labeled

16-10-06	Anti-Human IgG (H+L)	0.5 mg
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176-1006	Anti-Human IgG (H+L)	2.0 mg
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#### Labeled Streptavidin

474-3000	HRP-labeled	1.0 mL, liquid
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475-3000	AP-labeled	1.0 mL, liquid
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Catalog #	Description	Size
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### Substrates for Western Blotting

#### Phosphatase Colorimetric Substrates

50-81-18	BCIP/NBT Substrate	100 mL
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50-81-07	BCIP/NBT Substrate	600 mL
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50-81-30	FirePhos™ Membrane AP Substrate	100 mL
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50-81-40	FirePhos Membrane AP Substrate	400 mL
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50-81-34	FirePhos Membrane AP Substrate	1000 mL
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#### Phosphatase Chemiluminescent Substrates

55-60-03	PhosphaGLO™ AP Substrate	30 mL
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55-60-04	PhosphaGLO AP Substrate	100 mL
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55-60-01	PhosphaGLO Reserve AP Substrate	30 mL
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55-60-02	PhosphaGLO Reserve AP Substrate	100 mL
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#### Peroxidase Chromogenic Substrates

50-77-18	TMB Membrane Substrate	100 mL
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50-77-03	TMB Membrane Substrate	200 mL
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50-73-00	4 CN Substrate	600 mL
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50-73-04	4 CN Substrate	2700 mL
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#### Peroxidase Chemiluminescent Substrates

54-61-02	LumiGLO Chemiluminescent Substrate	60 mL
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54-61-00	LumiGLO Chemiluminescent Substrate	240 mL
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54-61-01	LumiGLO Chemiluminescent Substrate	720 mL
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54-71-00	LumiGLO Reserve Substrate Kit	2400 cm <sup>2</sup>
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54-71-01	LumiGLO Reserve Substrate Kit	600 cm <sup>2</sup>
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#### Assay Support Reagents

50-84-00	Coating Solution Concentrate	50 mL
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54-15-01	HRPStabilizer	200 mL
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55-15-00	APStabilizer	200 mL
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50-61-00	10% BSA Diluent/Blocking Solution Concentrate	200 mL
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50-82-01	Milk Diluent/Blocking Solution Concentrate	200 mL
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71-83-00	Detector™ Block (5X)	240 mL
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50-63-00	Wash Solution Concentrate (20X)	800 mL
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50-63-06	Biotin Wash Solution Concentrate (10X)	200 mL
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60-00-50	Biodyne® B Nylon Membrane	1 roll
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HSA=human serum adsorbed.

To order or for more information, call KPL at 800.638.3167, 301.948.7755, Fax: 301.948.0169. [www.kpl.com](http://www.kpl.com) or contact your local sales partner.



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